(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 August 2002 (22.08.2002)

PCT

(10) International Publication Number
WO 02/064148 A2

(51) International Patent Classification[7]: A61K 31/726, 31/727, 31/728, 31/737, 47/28, 47/44, A61P 7/02, 7/04

(21) International Application Number: PCT/JP02/01299

(22) International Filing Date: 15 February 2002 (15.02.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2001-40661 16 February 2001 (16.02.2001) JP
2001-299632 28 September 2001 (28.09.2001) JP

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/064148 A2

(54) Title: FORMULATION CONTAINING MUCOPOLYSACCHARIDE AND METHOD FOR PRODUCING THEREOF

(57) Abstract: An oral administrable enteric formulation of mucopolysaccharide is provided by using an absorption enhancer for mucopolysaccharide comprising of fatty acid having 6 to 14 carbon atoms or salt, ester and amide derivative thereof; steroid carboxylic acid, or salt, ester and amide derivative thereof; and aliphatic poly-basic acid, or salt, ester and amide derivative thereof; or mixture thereof. Said mucopolysaccharide has been only administered by the injection formulations, and the effective blood concentration of said mucopolysaccharide has not been obtained by oral administration.

DESCRIPTION

FORMULATION CONTAINING MUCOPOLYSACCHARIDE AND METHOD FOR PRODUCING THEREOF

TECHNICAL FIELD

The present invention relates to a formulation containing mucopolysaccharide, more specifically, to an oral administrable formulation containing mucopolysaccharide, which has not been administered orally, as well as the method for producing thereof.

BACKGROUND ART

Mucopolysaccharide such as heparin or low molecular weight heparin (LMWH) has no anticoagulation activity itself; however, shows the anticoagulation activity by combining with antithrombin III existing in blood to significantly increase antithrombin effect and anti-factor Xa effect.

These heparins have been clinically used for treating or preventing the venous thrombosis, cardiac infarction, pulmonary embolism, cerebral infarction, limbs arterial thromboembolism or operative/post-operative thrombotic infarct, as well as for treatment of disseminated intravascular coagulation (DIC). The heparins have also been clinically used for preventing the blood coagulation during the application of the extracorporeal circuit such as hemodialyzer or heart-lung machine, or the blood catheterization as well as at the time of blood transfusion and the blood test.

The heparins are administered by injection such as intravenous drip, bolus or intermittent intravenous injection, subcutaneous injection or intramuscular injection, and these methods for administration cause a pain or stress to the patients. Therefore, for preventing a deep venous thrombosis after hip joint or knee joint

implant operation, heparin or LMWH is subcutaneouslly administered for several days, and thereafter Warfarin® (Trade name) is orally administered to the patients. However, Warfarin® is difficult to use clinically because of taking a long time to show its pharmacological activity and long elimination half-life, and of having drug interactions between other drugs, as well as unstable pharmacokinetic behavior even in individual patient. Therefore, there have been an idea to administer heparins continuously by subcutaneous injection; however, this method is not practical for the patients due to the pain and bleeding at the injected region. For other administration methods of heparin, an ointment for transdermal preparation has been proposed; however the ointment formulation has not been replaced for Warfarin®. Furthermore, the development studies of an orally or transpulmonarilly administrable formulation have been investigated up to now. However, it has been well known that heparins are difficult to be absorbed from the gastrointestinal tract after oral administration due to its high molecular weight (M.W.: 2,000 to 20,000) and its strong negative charge (high sulfate degree).

It is reported that the heparins are useful for treatment of venous thromboembolism and ischemic cardiovascular diseases such as deep venous thrombosis, pulmonary embolism, stroke, unstable angina or acute cardiac infarction; however, clinical use of heparins are limited because the injection preparation is only practically available.

In case of Warfarin®, which is widely used for the anticoagulative therapy, the appearance of its pharmacological activity is very slow and therefore, it is necessary to take several days to reach the effective blood drug concentration after oral multiple administrations and also take a long time to be eliminated from the body after the cessation of drug administration. Furthermore, the blood Warfarin® concentrations show a wide inter-

and intra-subject variations. Therefore, the strict monitoring for administration of Warfarin® is necessary. Additionally, it is well known that Warfarin® is a typical drug which shows high drug-drug interactions because of high plasma protein binding, i.e., when Warfarin® is used with aspirin or phenylbutazone which is also highly bound to plasma protein, bleeding may occur due to the increase in free fraction of Warfarin® in the plasma.

Therefore, the effective oral anti-coagulation drugs instead of Warfarin® are strongly desired in this field.

The anti-coagulation effect of heparins increases after its intravenous or subcutaneous injection, and the elimination half-life of the drug from the blood circulation is short. Therefore, the orally administrable formulations of heparins have been proposed as an anticoagulant instead of Warfarin® due to its easy control of drug administration. For example, there are oral formulations of LMWH using BEODAS (high bio-degradable oral preparation) as a drug carrier proposed by Elan Corporation plc, Ireland, an oral formulation of heparin using Technosphere (delivery vehicle) as a drug carrier proposed by Pharmaceutical Discovery, Ltd., U.S.A., an orally formulation of heparin using sodium N-[8-(2-hydroxybenzoyl)amino]caprylate (*American Journal of Surgery*: Vol. 176, No. 2, 176-178, 1998; *Circulation*: Vol. 98, No. 16, 1610-1615, 1998) as a drug carrier, or an orally formulation of heparin using sodium N-[10-(2-hydroxybenzoyl)amino]decanoate (*Annals of Surgery*: Vol. 231, No. 6, 789-794, 2000) as a drug carrier by Emisphere Technologies, Inc., U.S.A. These formulations were designed to make heparin absorbable from the gastrointestinal tract using the carrier for heparin. However, these formulations are not available clinically because of the side effect such as emesis, or of large inter-subject variation of the absorption of heparin and so on.

There have been some other trials to develop non-injectable

formulations of heparin, and these are for example, non-stressed intrapulmonary administrable preparation using a nebulizer proposed by Inhale Therapeutic Systems, Inc., U.S.A. or transdermal preparation of spray gel nebulizer using poly dispersed liposome coat (spray-ben) proposed by Ratiopharm, Ltd., Germany. The former preparation is under pre-clinical stage and the latter preparation is limited to use for only surface phlebitis and not launched up to now.

The object of the present invention is to discover an effective, stable and safe oral administrable formulation of mucopolysaccharide such as heparin or LMWH.

The present inventors found out that it is necessary to use a large amount of mucopolysaccharide and absorption enhancer thereof to obtain an effective blood concentration using the above-mentioned drug carrier with mucopolysaccharide, and confirmed that these formulations are not practical formulations.

On the contrary, enteric formulation of mucopolysaccharide with absorption enhancer thereof can decrease the total amount of mucopolysaccharide and absorption enhancer. In this case, it was found that the mucopolysaccharide can be absorbed from the small intestine (i.e., duodenum, jejunum and ileum), mainly from jejunum and ileum, and the absorption efficiency of mucopolysaccharide slightly varied in accordance with the varieties of mucopolysaccharides or absorption enhancer thereof to be used. Therefore, it is advantageous to retain mucopolysaccharide in the absorption site, that is, the small intestine such as duodenum, jejunum and ileum, to obtain the effective blood concentration of the drug for a long period of time without any adverse action in the stomach or other digestive organs. Furthermore, it is also advantageous to use the mucopolysaccharide in the form of an aqueous solution or lyophilized powder thereof.

DISCLOSURE OF THE INVENTION

According to the present invention, there provided the followings:

(1) a formulation containing mucopolysaccharide and absorption enhancer thereof, wherein said formulation is characterized by its enteric formulation to release the mucopolysaccharide and absorption enhancer thereof from the formulation when said formulation reaches at the middle to lower part of small intestine;

(2) the formulation according to (1), wherein the said formulation contains mucosa adhesive substance which adheres to mucosal membrane of the middle to lower part of small intestine and retains there for a long period of time;

(3) the formulations according to (1) and (2), in which said mucosa adhesive substance is acidic polymer having carboxylic group or sulfonic group or salt thereof;

(4) the formulations according to (1) to (3) which comprises a basic layer having a drug container, a surface layer sealing said drug container and a drug-carrying layer for containing mucopolysaccharide and absorption enhancer thereof in which the basic layer consists of water-insoluble polymer, the surface layer consists of enteric substances, and the drug container consists of said basic layer and said surface layer;

(5) the formulations according to (1) to (4), in which said mucopolysaccharide is having the average molecular weight from 2,000 to 20,000;

(6) the formulations according to (1) to (5), in which said mucopolysaccharide is having the average molecular weight from 3,000 to 9,000;

(7) the formulations according to (1) to (6), in which said mucopolysaccharide is selected from the group consisting of heparin, low molecular weight heparin, hyaluronic acid, chondroitin sulfate, dermatan sulfate, heparan sulfate, ketaran sulfate, and

glucurono-2-amino-2-deoxyglycoglycan sulfate;

(8) the formulations according to (1) to (6), in which said mucopolysaccharide is heparin or low molecular weight heparin;

(9) the formulations according to (1) to (8), in which said formulation contains mucopolysaccharide in the form of aqueous solution;

(10) the formulations according to (1) to (9), in which said formulation contains mucopolysaccharide in the form of lyophilized powder thereof;

(11) the formulations according to (1) to (10), in which said absorption enhancer for mucopolysaccharide is selected from the group consisting of fatty acid having 6 to 14 carbon atoms, or salt, ester and amide derivative thereof; steroid carboxylic acid, or salt, ester and amide derivative thereof; and aliphatic poly-basic acid, or salt, ester and amide derivative thereof; or mixture thereof;

(12) the formulations according to (1) to (10), in which said absorption enhancer for mucopolysaccharide is selected from the group consisting of deoxycholic acid, capric acid, edetic acid, caprylocaproyl macrogolglycerides and lauroyl macrogol-32 glycerides;

(13) the formulations according to (1) to (10), in which said absorption enhancer for mucopolysaccharide is a mixture of caprylocaproyl macrogolglycerides and lauroyl macrogol-32 glycerides;

(14) the formulations according to (1) to (10), in which said absorption enhancer for mucopolysaccharide is a mixture of caprylocaproyl macrogolglycerides and citric acid;

(15) the formulations according to (1) to (10), in which said absorption enhancer for mucopolysaccharide is caprylocaproyl macrogolglycerides;

(16) the formulations according to (2) to (15), in which said mucosa adhesive substance is acrylic acid polymer or salt thereof;

(17) the formulations according to (1) to (16), wherein said formulation is characterized by its enteric formulation containing powdery mixture of mucopolysaccharide and absorption enhancer thereof;

(18) the formulations according to (1) to (16), wherein said formulation is tablet obtained by tableting of powdery mixture of mucopolysaccharide and absorption enhancer thereof, or by tableting of granules prepared from the powdery mixture of mucopolysaccharide and absorption enhancer thereof, and then enteric-coated;

(19) the formulations according to (1) to (16), wherein said formulation is capsule formulation containing granules prepared from the powdery mixture of mucopolysaccharide and absorption enhancer thereof and the resulting capsule is enteric-coated; and

(20) the formulations according to (1) to (16), wherein said formulation is enteric capsule formulation containing granules prepared from the powdery mixture of mucopolysaccharide and absorption enhancer thereof.

BEST MODE FOR CARRYING OUT THE INVENTION

The mucopolysaccharides used in the present invention are mucopolysaccharides which are unstable in the digestive organs such as stomach, or are not absorbed from the gastrointestinal tract. These are in general having the molecular weight from about 2,000 to 20,000, preferably from about 3,000 to 12,000, and more preferably from about 3,000 to 9,000. These are glycosaminoglycans represented by heparin, and the examples are hyaluronic acid, chondroitin sulfate, dermatan sulfate, heparan sulfate, ketaran sulfate, glucurono-2-amino-2-deoxyglycoglycan sulfate, and the like. These are available by extracted and purified from the organs of animals or enzymatic or chemical treatment such as sulfation, desulfation, acetylation, deacetylation, epimerization or amination of synthetic or natural oligoheteropolysaccharides. Further, low molecular

ones of these compounds obtained by enzymatic or chemical treatment, for example, parnaparin sodium, dalteparin sodium, enoxaparin sodium, tinzaparin sodium, reviparin sodium and the like may be included.

The absorption enhancers for mucopolysaccharide used in the present invention are, for example, fatty acid having 6 to 14 carbon atoms such as caproic acid or salts thereof, caprylic acid or salt thereof, capric acid or salt thereof, lauric acid or salt thereof, hydrogenated caster oil, and esters or amide derivatives thereof; sodium N-[8-(2-hydroxybenzoyl)amino]caprylate, sodium N-[10-(2-hydroxybenzoyl)amino]decanoate and the like. Further, these are for example, steroid carboxylic acid or salt thereof as well as ester or amide derivative thereof such as deoxycholic acid, desoxycholic acid, chenodeoxycholic acid, ursodeoxycholic acid, taurocholic acid and the like; citric acid or salt thereof; aliphatic poly-basic acid such as disodium edetate as well as esters or amide derivatives thereof, and the like. These absorption enhancer for mucopolysaccharides can be used alone or combined together, for example, caprylocaproyl macrogol glycerides (*Supplement 2,000 of the European Pharmacopeia*) or Labrasol® (Trade name; Gattefossé, Ltd.), lauroyl macrogol-32 glycerides (*Supplement of European Pharmacopeia*) or Gelucire® 44/14 (Trade name; Gattefossé, Ltd.), stearoyl macrogol glycerides (*Supplement of European Pharmacopeia*) or Gelucire® 50/13 (Trade name; Gattefossé, Ltd.). Further, the mixture of caprylocaproyl macrogol glycerides or Labrasol® with an organic acid such as citric acid, sulfuric acid, capric acid, lauroyl macrogol-32 glycerides, sodium laury sulfate, d-α-tocopheryl polyethylene glycol 1000 succinate, polyethylene (80) sorbitan monooleate, polyoxyethylene hydrogenated castor oil 60 and the like, can be used as the absorption enhancer for mucopolysaccharide.

These mucopolysaccharide and absorption enhancer thereof are protected by the enteric substance, which is insoluble in the stomach and is soluble in the small intestine, to prevent an inactivation in the oral cavity, esophagus or stomach. Examples of these enteric substances are hydroxypropyl methylcellulose phthalate (*Japanese Pharmacopoeia, the XIII Ed.*), hydroxypropyl methylcellulose acetate succinate (Shin-Etsu Chemical, Co. Ltd.), carboxymethyl ethyl cellulose (Froint Ind., Ltd.), cellulose acetate phthalate (*Japanese Pharmacopoeia, the XIII Ed.*), methacrylic acid-ethylacrylate copolymer (Trade name: Eudragit® L-100-55; Rohm Pharm, Ltd.), methacrylic acid-methylmethacrylate copolymer (Trade name: Eudragit® L-100, S-100; Rohm Pharm, Ltd.), methacrylic acid copolymer-L (Trade name: Eudragit® L; Rohm Pharm, Ltd.), methacrylic acid copolymer-LD (Trade name: Eudragit® LD; Rohm Pharm, Ltd.), methacrylic acid copolymer-S (Trade name: Eudragit® S; Rohm Pharm, Ltd.), aminoalkyl methacrylate copolymer-E (Trade name: Eudragit® E; Rohm Pharm, Ltd.) and the like. These enteric substances may be dissolved at the desired intestinal site from duodenum to lower part of the small intestine by varying the types and combination of the enteric substances, quantities thereof, thickness of the membrane layer, and the like. Further, the release rate of drug from the formulation may be controlled by using the water-insoluble pharmaceutical polymer such as ethyl cellulose, aminoalkyl methacrylate copolymer, cellulose acetate, chitin and chitosan, as well as by combining the above-mentioned enteric substances at the desired site in the digestive tract.

These enteric formulations are preferably retaining at the absorption site for a long period of time. The adhesive substance, such as described in the Japanese Patent Laid-open Hei 5-132416; Hei 11-130696; and *Pharmaceutical Research*, Vol. 12, 397-405, may be used for these purposes. These adhesive substances may be

pharmaceutically acceptable ones, and adhere to the mucous membrane after absorbing a water to manifest the viscosity. It is most preferable to use the adhesive substances that swell with water and manifest the high viscosity. Such adhesive substances are natural or synthetic polymers, for example, non water-insoluble straight-chained polysaccharide such as Curdlan (Curdlan N, Food additive), mucin, agar, gelatin, pectin, carrageenan, sodium alginate, locust bean gum, xanthan gum, tragacanth gum, chitosan, pullulan, waxy corn starch, sucralfate, cellulose, and derivatives thereof such as hydroxypropyl cellulose, hydroxypropyl methyl cellulose and the like. The synthetic viscous polymers such as acidic polymer having carboxylic group, sulfonic group or salt thereof are preferably used, and acrylic acid polymer or salt thereof is most preferably used. For example, these are carbomer (carbopol® 940, 934, 941, 1342, 974 etc.; Trade name; The BF Goodrich, Ltd.), Hiviswako® 103, 104, 105 (Wako Pure Chemical Industries, Ltd.), NOVEON AAI (The BF Goodrich, Ltd.), and calcium polycarbophil (*U. S. Pharmacopoeia XXIII*). These adhesive substances effect so as to retain at the small intestine or adhere at the mucous membrane of small intestine by absorbed water and manifested viscosity when the drugs reach at the absorption site, that is, from duodenum to small intestine. For this case, it may be easily obtained by combining the adhesive substances with the mucopolysaccharide and the absorption enhancer thereof, or coated the mucopolysaccharide and the absorption enhancer thereof with the enteric substances, as well as admixed the adhesive substances with the enteric substances.

The common additive used for the pharmaceutical formulations may be added to the adhesive substances containing mucopolysaccharide and absorption enhancer thereof, if necessary. These are for example, binders such as crystalline cellulose, corn starch, starch, glucose, gelatin, methyl cellulose, carboxymethyl cellulose,

hydroxypropyl cellulose, hydroxymethyl cellulose, dextrin, and the like; disintegrator such as carboxymethyl cellulose calcium, low substituted hydroxypropyl cellulose, and the like; surfactants; anti-acid agents such as magnesium aluminate metasilicate, light anhydrous silicic acid, anhydrous dibasic calcium phosphate, magnesium hydroxide and the like; mucosa protecting agents; and adsorbents. The effect of absorption of drugs can be increased by the presence of glycerin ester or poly-glycerin ester of fatty acid to prolong the retention time in the digestive tract or to sustain the release of the mucopolysaccharide from the formulation.

The amount of mucopolysaccharide for the formulation of the present invention may be, for example, from 0.2 to 100 mg/kg, more preferably from 0.5 to 10 mg/kg. The amount of absorption enhancer for mucopolysaccharide may also be, for example, from 0.2 to 100 mg/kg, more preferably from 0.5 to 10 mg/kg. The present inventors found out that the mucopolysaccharide was rapidly absorbed into the systemic blood through the intestinal wall using an absorption enhancer such as caprylocaproyl macrogolglycerides in the presence of water. Therefore, it is preferable to use an aqueous solution containing mucopolysaccharide alone or with absorption enhancer together for preparing the pharmaceutically formulations. The aqueous solution containing mucopolysaccharide can be formulated directly or indirectly after absorbing with the pulverization agent in order to form the powder. Accordingly, these mucopolysaccharide and absorption enhancer thereof are formulated to granules, fine granules or tablets with the enteric substances or other additives, if necessary, by common methods, and further coated with adhesive substances or enteric substances, if necessary. The granules or fine granules can be filled in the common capsule and the resulting capsule is enteric-coated, or further filled in another enteric capsule. The granules or fine granules can also be tableted to

prepare the tablet formulations.

Furthermore, the granules or fine granules can be formulated to three layers micro or mill capsule disclosed in Patent Gazette WO 00/32172. The three layers micro or mill capsule consists of the basic layer having the drug container, the drug-carrying layer for containing a drug and further absorption enhancer if necessary, and the surface layer sealing the drug container and retaining the formulation in the desired absorption site for a long period of time. The basic layer, having the cavity containing the drug therein, consists of water-insoluble polymer to prevent the permeation of hydrolytic enzymes into the drug container. The water-insoluble polymer consisting of the basic layer may be, for example, ethyl cellulose, aminoalkylmethacrylate copolymer, cellulose acetate, chitin, chitosan and the like. These are used in alone or combined together. The drug container consisting of water-insoluble polymer is prepared by using a mold having regular or irregular hollow, or the film of water-insoluble polymer is spread on a metal mold having many projections and heated.

The drug-carrying layer contains mucopolysaccharide and absorption enhancer thereof, and consists of optional additives or mucosa adhesive substances and the like. The examples of additives or mucosa adhesive substances are polymers or gums such as carboxyvinyl polymer, polyacrylic acid/octyl acrylate copolymer, 2-ethylhexyl acrylate/vinyl pyrrolidone copolymer, acrylic acid/silk-fibroin copolymer resin, macrogol, methyl acrylate/2-ethylhexyl acrylate copolymer resin, gum arabic, polyvinyl alcohol, polyvinylpyrrolidone, methyl-cellulose, hydroxypropyl-methyl cellulose, polyisoprene, polyacrylic acid, sodium polyacrylate, alginic acid, α-starch, carboxymethylethyl cellulose, sodium carboxymethyl starch, crystalline cellulose, cyclodextrin, and the like. These are used alone or combined together and filled in the aforementioned water-insoluble drug container in the form

of fine powders, granules or solvent.

The surface layer seals the drug container and affects the absorption of the drug so as to retain the intestinal absorption site from the duodenum to the ileum for a long period of time. The enteric substances such as hydroxypropyl methylcellulose phthalate (HP-55), methacrylic acid copolymer L (Eugragit® L), methacrylic acid copolymer LD (Eugragit® LD), methacrylic acid copolymer S (Eugragit® S), aminoalkylmethacrylate copolymer E (Eugragit® E), and the like can be used for this purpose. For example, the surface layer can be made by dissolving 225 mg of HP-55 (Shin-Etsu Chemical Ind., Ltd.) and 25 μl of triethyl citrate in 5 mL of a mixture solution of methylene chloride and methanol (4:1), and the resulting solution is cast on a Teflon® plate to form a film having from approximately 30 to 50 μm of thickness, and the obtained film seals the open area of the drug container. Further, the surface film layer can be also prepared from the solution of 225 mg of Eudragit® S100 or Eudragit® L100 and 150 μl of triethyl citrate in 5 mL of a mixture solution of methylene chloride and methanol (1:1).

These three layers micro- or milicapsules are administered orally themselves or filled into capsules or the enteric capsules and administered orally. After oral administration, mucopolysaccaride as an active ingredient is retained at the absorption site of small intestine and the excellent absorption of the drug can be obtained.

The followings are the preferable mucopolysaccharide formulations of the present invention.

Following solution preparation (1) or (2) is prepared and used for the formulations.

(1) Solution preparation containing mucopolysaccharide and absorption enhancer thereof with water.

(2) Solution preparation containing mucopolysaccharide and absorption enhancer thereof without water.

1. Capsule Formulation:

1) Filling with the solution preparation directly:

The solution preparation (1) or (2) is filled into capsule and then the capsule is enteric-coated, or filled into capsule made by enteric substance.

2) Filling with powder prepared from the solution preparation:

The solution preparation (1) or (2) is absorbed with the pulverization agent (such as, magnesium aluminate metasilicate, light anhydrous silicic acid, anhydrous dibasic calcium phosphate and the like) in order to form the powder, and resulting powder is filled into capsule then the capsule is enteric-coated, or filled into capsule made by enteric substance.

3) Filling with granules prepared from the solution preparation:

The solution preparation (1) or (2) is absorbed with the pulverization agent in order to form the powder, and resulting powder is granulated, and

(a) the obtained granules are filled into capsule then the capsule is enteric-coated, or filled into capsule made by enteric substance; or

(b) the obtained granules are further coated with enteric substance and filled into capsule.

2. Tablet Formulation:

1) The powder obtained in the above 1-2) is tableted to form the tablet with other excipients if necessary, and the obtained tablet is enteric-coated.

2) The granules obtained in the above 1-3) are tableted to form the tablet with other excipients if necessary, and the obtained tablet is enteric-coated.

3. Granules Formulation:

The granules obtained in the above 1-3) are coated with the enteric substance.

EXAMPLES

The present invention will be further illustrated by the following examples. It is to be understood that the present invention is not limited to these examples.

Experimental Example A

The following Solution Preparations (1) to (10) were prepared.

Solution Preparation (1):

1 L of phosphate buffered solution (pH 6.8) was made from 6.8 g of potassium hydrogen sulfate, 0.95 g of sodium hydroxide and purified water. Then, 1 mL of the Solution Preparation (1) was prepared by dissolving of 30 mg of parnaparin sodium and 5 mg of sodium salt of deoxycholic acid in the above obtained phosphate buffer (pH 6.8).

Solution Preparation (2):

3 mL of the Solution Preparation (2) was prepared by dissolving 90 mg of parnaparin sodium and 30 mg of sodium salt of deoxycholic acid in phosphate buffer (pH 6.8).

Solution Preparation (3):

3 mL of the Solution Preparation (3) was prepared by dissolving 90 mg of parnaparin sodium and 100 mg of sodium salt of capric acid in phosphate buffer (pH 6.8).

Solution Preparation (4):

3 mL of the Solution Preparation (4) was prepared by dissolving 90 mg of parnaparin sodium and 100 mg of sodium edetate in phosphate buffer (pH 6.8).

Solution Preparation (5):

3 mL of the Solution Preparation (5) was prepared by dissolving

90 mg of parnaparin sodium and 100 mg of sodium salt of taurocholic acid in phosphate buffer (pH 6.8).

Solution Preparation (6):

3 mL of the Solution Preparation (6) was prepared by dissolving 90 mg of parnaparin sodium and 100 mg of citric acid in phosphate buffer (pH 6.8).

Solution Preparation (7):

3 mL of the Solution Preparation (7) was prepared by dissolving 90 mg of parnaparin sodium and 100 mg of sodium lauryl sulfate in phosphate buffer (pH 6.8).

Solution Preparation (8):

3 mL of the Solution Preparation (8) was prepared by dissolving 90 mg of parnaparin sodium, 50 mg of sodium salt of capric acid and 50 mg of sodium edetate in phosphate buffer (pH 6.8).

Solution Preparation (9):

3 mL of the Solution Preparation (9) was prepared by dissolving 90 mg of parnaparin sodium and 2 mL of caprylocaproyl macrogolglycerides (EP: 1184) in phosphate buffer (pH 6.8).

Solution Preparation (10):

3 mL of the Solution Preparation (10) was prepared by dissolving 90 mg of parnaparin sodium, and 90 μl, 300 μl or 900 μl of caprylocaproyl macrogolglycerides (EP: 1184), respectively, in phosphate buffer (pH 6.8).

The following experiments were conducted using the Solution Preparation (1) to (10) prepared above.

Experiment 1:

The Solution Preparation (1) obtained above was administered into the duodenum of male SD rats weighing from 250 to 350 g at the dose volume of 1 mL/kg. As a reference, 1 mL of the Reference Solution containing 30 mg/mL of parnaparin sodium was administered according to the same manner. The blood samples were collected from the left jugular vein of rats at 30, 60 and 120 minutes after the administration, respectively, and plasma anti-factor Xa activities were measured as the index of the absorbability of parnaparin sodium.

The results are shown in Table 1.

Table 1: Plasma anti-factor Xa activities after administration of parnaparin sodium (30 mg/kg) plus absorption enhancer thereof to rat intestine

Absorption enhancer	Dose	Administration site	Time (min.) after administration		
			30	60	120
None	-	Duodenum	-	-	-
Na salt of Deoxycholic acid	5mg/kg	Duodenum	+	-	-

0.1U/mL ≦ Anti-factor Xa activity < 0.2U/mL : +
0.1U/mL > Anti-factor Xa activity : -

As apparently shown in Table 1, a slight increase in anti-factor Xa activity was observed at 30 minutes after the administration of the Solution Preparation (1) containing sodium salt of deoxycholic acid as the absorption enhancer of parnaparin sodium; however, no increase in anti-factor Xa activity was observed in case of Reference Solution.

Experiment 2:

The Solution Preparations (2) to (9) obtained above were administered at the duodenum of male SD rats weighing from 250 to 350 g at the dose volume of 1 mL/kg. The blood samples were collected from the jugular vein of rats at 15, 30 and 60 minutes after the

administration, and plasma anti-factor Xa activities in plasma were measured as the index of the absorbability of parnaparin sodium.

The results are shown in Table 2.

Table 2: Plasma anti-factor Xa activities after administration of parnaparin sodium (30 mg/kg) plus absorption enhancer thereof to rat intestine

Absorption enhancer	Dose (mg/kg)	Administration site	Time (min.) after administration		
			15	30	60
Na salt of Deoxycholic acid	10	Duodenum	+++	+++	++
Na salt of Capric acid	33.3	Duodenum	+++	+++	+++
Sodium Edetate	33.3	Duodenum	++	+++	+++
Na salt of Taurocholic acid	33.3	Duodenum	+	-	-
Citric acid	33.3	Duodenum	++	++	+
Sodium lauryl sulfate	33.3	Duodenum	++	++	++
Na salt of Capric acid/ Sodium Edetate	16.7 16.7	Duodenum	++	++	++
Caprylocaproyl macrogolgylcerides	667 µL/kg	Duodenum	+++	+++	+++

0.5U/mL ≦ Anti-factor Xa activity : +++
0.2U/mL ≦ Anti-factor Xa activity < 0.5U/mL : ++
0.1U/mL ≦ Anti-factor Xa activity < 0.2U/mL : +
0.1U/mL > Anti-factor Xa activity : -

As apparently shown in Table 2, more than 0.5 U/mL of high plasma anti-factor Xa activity was observed by the administration of the solution preparations containing sodium salt of deoxycholic acid, sodium salt of capric acid, sodium edetate and caprylocaproyl macrogolgylcerides as the absorption enhancer of parnaparin sodium, and more than 0.2 U/mL of moderate anti-factor Xa activity was observed by the administration of the solution preparations containing citric cid, sodium lauryl sulfate and a mixture of sodium salt of capric acid and sodium edetate as the absorption enhancer of parnaparin sodium. Further, less than 0.2 U/mL of weak anti-factor Xa activity was observed by the administration of the solution preparations containing sodium lauryl sulfate as the absorption enhancer of parnaparin sodium.

Experiment 3:

The Solution Preparations (2), (3), (4) and (9) obtained above were administered at the duodenum, jejunum or ileum of male SD rats weighing from 250 to 350 g at the dose volume of 1 mL/kg of the preparation. The blood samples were collected from the jugular vein of rats at 15, 30, 60, 120 and 240 minutes after the administration, and plasma anti-factor Xa activities were measured as the index of the absorbability of parnaparin sodium.

The results are shown in Table 3.

Table 3: Plasma anti-factor Xa activities after administration of parnaparin sodium (30 mg/kg) plus absorption enhancer thereof to rat intestine

Absorption enhancer	Dose (mg/kg)	Administration Site	Time (min.) after administration				
			15	30	60	120	240
Na salt of Deoxycholic acid	10	Duodenum	+++	+++	++	++	-
		Jejunum	+++	+++	+++	+++	++
		Ileum	+++	+++	++	-	-
Na salt of Capric acid	33.3	Duodenum	+++	+++	+++	++	-
		Jejunum	+++	+++	+++	+++	+
		Ileum	+++	+++	+++	+++	++
Sodium Edetate	33.3	Duodenum	++	++	+++	++	-
		Jejunum	+	++	+++	++	-
		Ileum	+	++	+++	+++	-
Caprylocaproyl Macrogolgylcerides	667 µL/kg	Duodenum	+++	+++	+++	+++	++
		Jejunum	+++	+++	+++	+++	+++
		Ileum	+++	+++	+++	+++	+++

0.5U/mL ≦ Anti-factor Xa activity : +++
0.2U/mL ≦ Anti-factor Xa activity < 0.5U/mL : ++
0.1U/mL ≦ Anti-factor Xa activity < 0.2U/mL : +
0.1U/mL > Anti-factor Xa activity : -

As clearly shown in Table 3, in case of the administration of the solution preparation containing sodium salt of deoxycholic acid as an absorption enhancer of parnaparin sodium, the highest anti-factor Xa activity was observed by the administration of said preparation at the jejunum (middle part of the small intestine). In case of the administration of the solution preparation containing

sodium salt of capric acid as an absorption enhancer of parnaparin sodium, the highest anti-factor Xa activity was observed by the administration of said preparation at the ileum (lower part of the small intestine). Further, in case of the administration of the solution preparation containing sodium edetate as an absorption enhancer of parnaparin sodium, the highest anti-factor Xa activity was observed by the administration of said preparation at the duodenum, jejunum and ileum 60 minutes after the administration, and in case of the administration of the solution preparation containing caprylocaproyl macrogolgylcerides as an absorption enhancer of parnaparin sodium, the high anti-factor Xa activities were observed after the administration to the middle and lower parts of small intestine, that is at the jejunum and ileum.

Experiment 4:

The Solution Preparation (10) obtained above was administered at jejunum or ileum of male SD rats weighing from 250 to 350 g at the dose volume of 1 mL/kg. The blood samples were collected from the jugular vein of rats at 15, 30, 60, 120 and 240 minutes after the administration, and plasma anti-factor Xa activities were measured as the index of the absorbability of parnaparin sodium.

The results are shown in Table 4.

Table 4: Plasma anti-factor Xa activities after administration of parnaparin sodium (30 mg/kg) plus absorption enhancer thereof to rat intestine

Absorption enhancer	Dose (μL/kg)	Administration Site	Time (min.) after administration				
			15	30	60	120	240
Caprylocaproyl Macrogolgylcerides	30	Jejunum	+++	+++	+++	+	-
		Ileum	+++	+++	++	++	-
	100	Jejunum	+++	+++	+++	+++	++
		Ileum	+++	+++	+++	+++	-
	300	Jejunum	+++	+++	+++	+++	-
		Ileum	+++	+++	+++	+++	++

0.5U/mL ≦ Anti-factor Xa activity : +++
0.2U/mL ≦ Anti-factor Xa activity < 0.5U/mL : ++
0.1U/mL ≦ Anti-factor Xa activity < 0.2U/mL : +
0.1U/mL > Anti-factor Xa activity : -

As apparently shown in Table 4, in case of the administration of the Solution Preparation (10) containing caprylocaproyl macrogolgylcerides as an absorption enhancer of parnaparin sodium, the anti-factor Xa activities plasma increased dose-dependently.

Experimental Example B

The following Solution Preparations (11) to (20) were prepared.

Solution Preparation (11):

60 mg of parnaparin sodium and 0.5 mg of citric acid were dissolved in 0.33 mL of purified water, and to this solution was added 0.67 mL of caprylocaproyl macrogolglycerides and the mixture was well stirred. Then 60 mg of sodium lauryl sulfate was further added to this solution and the mixture was well stirred. After stirring for 45 to 60 minutes, the clear Solution Preparation (11) was obtained.

Solution Preparation (12):

60 mg of parnaparin sodium and 1.5 mg of citric acid were dissolved in 0.33 mL of purified water, and to this solution was added 0.67 mL of caprylocaproyl macrogolglycerides and the mixture was well stirred. Then 0.05 mL of polyoxyethylene (80) sorbitanmonooreate was further added to this solution and the mixture was well stirred. After stirring for 45 to 60 minutes, the clear Solution Preparation (12) was obtained.

Solution Preparation (13):

60 mg of parnaparin sodium and 2.0 mg of citric acid were dissolved in 0.33 mL of purified water, and to this solution was

added 0.67 mL of caprylocaproyl macrogolglycerides and the mixture was well stirred. Then 50 mg of polyoxyethylene hydrogenated castor oil 60 was further added to this solution and the mixture was well stirred. After stirring for 45 to 60 minutes, the clear Solution Preparation (13) was obtained.

Solution Preparation (14):

60 mg of parnaparin sodium and 2.0 mg of citric acid were dissolved in 0.33 mL of purified water, and to this solution was added 0.67 mL of caprylocaproyl macrogolglycerides and the mixture was well stirred. Then 100 mg of lauroyl macrogol-32 glycerides (EP) was further added to this solution and the mixture was well stirred. After stirring for 45 to 60 minutes, the clear Solution Preparation (14) was obtained.

Solution Preparation (15):

60 mg of parnaparin sodium was dissolved in 0.33 mL of purified water (solution A). Separately, 20 mg of capric acid was added to 0.67 mL of caprylocaproyl macrogolglycerides and the mixture was well stirred (solution B). Then, the solution A was added to the solution B, and the mixture was well stirred, and to this mixture was added 50 mg of sodium lauryl sulfate and the resulting mixture was well stirred. After stirring for 45 to 60 minutes, the clear Solution Preparation (15) was obtained.

Solution Preparation (16):

60 mg of parnaparin sodium and 1.5 mg of citric acid were dissolved in 0.33 mL of purified water, and to this solution was added 0.47 mL of caprylocaproyl macrogolglycerides and the mixture was well stirred. Then 200 mg of d-α-tocopheryl polyethylene glycol 1000 succinate was further added to this solution and the mixture was well stirred. After stirring for 45 to 60 minutes, the clear

Solution Preparation (16) was obtained.

Solution Preparation (17):

60 mg of parnaparin sodium and 1.5 mg of citric acid were dissolved in 0.33 mL of purified water, and to this solution was added 0.47 mL of caprylocaproyl macrogolglycerides and the mixture was well stirred. Then 30 mg of sodium lauryl sulfate and 200 mg of d-α-tocopheryl polyethylene glycol 1000 succinate were added and the mixture was well stirred. After stirring for 45 to 60 minutes, the clear Solution Preparation (17) was obtained.

Solution Preparation (18):

60 mg of parnaparin sodium and 1.5 mg of citric acid were dissolved in 0.67 mL of purified water, and to this solution was added 0.33 mL of caprylocaproyl macrogolglycerides and the mixture was well stirred. After stirring for 45 to 60 minutes, the clear Solution Preparation (18) was obtained.

Solution Preparation (19):

60 mg of parnaparin sodium and 2.0 mg of citric acid were dissolved in 0.67 mL of purified water, and to this solution was added 0.33 mL of caprylocaproyl macrogolglycerides and the mixture was well stirred. Then 100 mg of lauroyl macrogol-32 glycerids (EP) was added and the mixture was well stirred. After stirring for 45 to 60 minutes, the clear Solution Preparation (19) was obtained.

Solution Preparation (20):

90 mg of parnaparin sodium and 2.0 mg of citric acid were dissolved in 0.33 mL of purified water, and to this solution was added 0.67 mL of caprylocaproyl macrogolglycerides and the mixture was well stirred. Then 100 mg of lauroyl macrogol-32 glycerids (EP) was added and the mixture was well stirred. After stirring for 45

to 60 minutes, the clear Solution Preparation (20) was obtained.

The following experiments were conducted using the Solution Preparations (11) to (20) prepared above.

Experiment 5:

167 μL/kg of the Solution Preparations (11) to (16) obtained above were administered into the ileum of male Wistar rats weighing from 280 to 480 g so as to administer 10 mg/kg of parnaparin sodium. The blood samples were collected from the jugular vein of rats before the administration and 15, 30, 60, 120 and 240 minutes after the administration, and anti-factor Xa activities in plasma were measured as the index of the absorbability of parnaparin sodium.

The results are shown in Table 5.

Table 5: Plasma anti-factor Xa activities after administration to rat intestine

Preparation No.	Dose of Parnaparin	Administration site	Time (min.) after administration					
			0	15	30	60	120	240
(11)	10 mg/kg	ileum	-	+++	+++	+++	++	++
(12)			-	+++	+++	+++	+++	+++
(13)			-	+++	+++	+++	+++	+++
(14)			-	+++	+++	+++	+++	+++
(15)			-	+++	+++	+++	+++	++
(16)			-	+++	+++	+++	+++	++

0.5U/mL ≦ Anti-factor Xa activity : +++
0.2U/mL ≦ Anti-factor Xa activity < 0.5U/mL : ++
0.1U/mL > Anti-factor Xa activity : -

As apparently shown in Table 5, in cases of the administration of the Solution Preparations (11) to (16), more than 0.5 U/mL of high anti-factor Xa activities in plasma were observed.

Experiment 6:

83 μL/kg of the Solution Preparations (13), (14) and (17) were administered into the ileum of male Wistar rats weighing from 330 to 640 g so as to administer 5 mg/kg of parnaparin sodium. The

blood samples were collected from the jugular vein of rats before the administration and 15, 30, 60, 120 and 240 minutes after the administration, and anti-factor Xa activities in plasma were measured as the index of the absorbability of parnaparin sodium.

The results are shown in Table 6.

Table 6: Plasma anti-factor Xa activities after administration to rat intestine

Preparation No.	Dose of Parnaparin	Administration site	Time (min.) after administration					
			0	15	30	60	120	240
(13)	5 mg/kg	ileum	-	+++	+++	+++	+++	+++
(14)			-	+++	+++	+++	+++	+++
(17)			-	+++	+++	++	+	-

0.5U/mL ≦ Anti-factor Xa activity : +++
0.2U/mL ≦ Anti-factor Xa activity < 0.5U/mL : ++
0.1U/mL ≦ Anti-factor Xa activity < 0.2U/mL : +
0.1U/mL > Anti-factor Xa activity : -

As apparently shown in Table 6, in cases of the administration of the Solution Preparations (13), (14) and (17), more than 0.5 U/mL of high anti-factor Xa activities in plasma were observed.

Experiment 7:

33 μL/kg of the Solution Preparations (14), (18) and (19), and 22 μL/kg of the Solution Preparations (20) were administered into the ileum of male Wistar rats weighing from 330 to 640 g so as to administer 2 mg/kg of parnaparin sodium. The blood samples were collected from the jugular vein of rats before the administration and 15, 30, 60, 120 and 240 minutes after the administration, and anti-factor Xa activities in plasma were measured as the index of the absorbability of parnaparin sodium.

The results are shown in Table 7.

Table 7: Plasma anti-factor Xa activities after administration to rat intestine

Preparation No.	Dose of Parnaparin	Administration Site	Time (min.) after administration					
			0	15	30	60	120	240
(14)	2 mg/kg	ileum	-	++	++	+++	++	-
(18)			-	+++	+++	+++	+++	++
(19)			-	++	+++	+++	++	-
(20)			-	+++	+++	+++	+++	+

0.5U/mL ≦ Anti-factor Xa activity : +++
0.2U/mL ≦ Anti-factor Xa activity < 0.5U/mL : ++
0.1U/mL ≦ Anti-factor Xa activity < 0.2U/mL : +
0.1U/mL > Anti-factor Xa activity : -

As apparently shown in Table 7, in cases of the administration of the Solution Preparations (14), (18), (19) and (20), more than 0.5 U/mL of high anti-factor Xa activities in plasma were observed.

Experiment 8:

17 μL/kg of the Solution Preparations (13), (14) and (17) were administered into the ileum of male Wistar rats weighing from 330 to 640 g so as to administer 1 mg/kg of parnaparin sodium. The blood samples were collected from the jugular vein of rats before the administration and 15, 30, 60, 120 and 240 minutes after the administration, and anti-factor Xa activities in plasma were measured as the index of the absorbability of parnaparin sodium.

The results are shown in Table 8.

Table 8: Plasma anti-factor Xa activities after administration to rat intestine

Preparation No.	Dose of Parnaparin	Administration Site	Time (min.) after administration					
			0	15	30	60	120	240
(13)	1 mg/kg	ileum	-	+	++	+	+	-
(14)			-	++	++	+	+	+
(17)			-	++	++	+	+	+

0.2U/mL ≦ Anti-factor Xa activity < 0.5U/mL : ++
0.1U/mL ≦ Anti-factor Xa activity < 0.2U/mL : +
0.1U/mL > Anti-factor Xa activity : -

As apparently shown in Table 8, in cases of the administration

of the Solution Preparations (13), (14) and (17), less than 0.3 U/mL of weak anti-factor Xa activities in plasma were observed.

Experimental Example C

The following Solution Preparations (21) to (25) were prepared.

Solution Preparation (21):

A mixture solution of 1 g of parnaparin sodium in total amount of 50 mL of purified water was well stirred and lyophilized at -10°C for 20 hours, -45°C for 10 hours, 0°C for 40 hours, and 40°C for 20 hours, respectively. Then, the resulting powder was screened with sieve #60 to obtain the lyophilized powder.

On the contrary, 125 mg of lauroyl macrogol-32 glycerides was added to 0.8 mL of caprylocaproyl macrogolglycerides, and the mixture was well stirred under 50°C to obtain the clear solution. To this solution was added 15 mg of the above obtained lyophilized powder, and the mixture was well stirred to obtain the Solution Preparation (21).

Solution Preparation (22):

A mixture solution of 1 g of parnaparin sodium in total amount of 100 mL of purified water was well stirred and lyophilized under the same conditions described above, and the resulting powder was screened with sieve #60 to obtain the lyophilized powder.

Then, 125 mg of lauroyl macrogol-32 glycerides was added to 0.8 mL of caprylocaproyl macrogolglycerides, and the mixture was well stirred under 50°C to obtain the clear solution. To this solution was added 15 mg of the above obtained lyophilized powder, and the mixture was well stirred to obtain the Solution Preparation (22).

Solution Preparation (23):

A mixture solution of 0.1 g of parnaparin sodium in total amount of 100 mL of purified water was well stirred and lyophilized under the same conditions described above, and the resulting powder was screened with sieve #60 to obtain the lyophilized powder.

Then, 125 mg of lauroyl macrogol-32 glycerides was added to 0.8 mL of caprylocaproyl macrogolglycerides, and the mixture was well stirred under 50°C to obtain the clear solution. To this solution was added 15 mg of the above obtained lyophilized powder, and the mixture was well stirred to obtain the Solution Preparation (23).

Solution Preparation (24):

A mixture solution of 0.5 g of parnaparin sodium and 0.5 g of mannitol in total amount of 10 mL of purified water was well stirred and lyophilized under the same conditions described above, and the resulting powder was screened with sieve #60 to obtain the lyophilized powder.

Then, 125 mg of lauroyl macrogol-32 glycerides was added to 0.8 mL of caprylocaproyl macrogolglycerides, and the mixture was well stirred under 50°C to obtain the clear solution. To this solution was added 30 mg of the above obtained lyophilized powder, and the mixture was well stirred to obtain the Solution Preparation (24).

Solution Preparation (25):

A mixture solution of 0.5 g of parnaparin sodium and 0.5 g of sucrose in total amount of 10 mL of purified water was well stirred and lyophilized under the same conditions described above, and the resulting powder was screened with sieve #60 to obtain the lyophilized powder.

Then, 125 mg of lauroyl macrogol-32 glycerides was added to 0.8 mL of caprylocaproyl macrogolglycerides, and the mixture was

well stirred under 50°C to obtain the clear solution. To this solution was added 30 mg of the above obtained lyophilized powder, and the mixture was well stirred to obtain the Solution Preparation (25).

Experiment 9:

107 μL/kg of the Solution Preparations (21) to (25) obtained above were administered into the ileum of male Wistar rats weighing from 430 to 470 g so as to administer 2 mg/kg of parnaparin sodium. The blood samples were collected from the jugular vein of rats before the administration and 15, 30, 60, 120 and 240 minutes after the administration, and anti-factor Xa activities in plasma were measured as the index of the absorbability of parnaparin sodium.

The results are shown in Table 9.

Table 9: Plasma anti-factor Xa activities after administration to rat intestine

Preparation No.	Dose of Parnaparin	Administration Site	Time (min.) after administration					
			0	15	30	60	120	240
(21)	2 mg/kg	Ileum	-	++	++	++	+	+
(22)			-	++	++	++	+	-
(23)			-	++	++	++	++	+
(24)			-	++	++	++	++	+
(25)			-	++	+++	+++	++	++

0.5U/mL ≦ Anti-factor Xa activity : +++
0.2U/mL ≦ Anti-factor Xa activity < 0.5U/mL : ++
0.1U/mL > Anti-factor Xa activity : -

Experimental Example D

The enteric capsules obtained by Example 2 were orally administered to three beagle dogs (Dog IDs: S, G and Y) weighing from 9.5 to 11.5 kg at the dose of three capsules per dog. The blood samples were collected from the left jugular vein before the administration, and 1, 2, 3, 4, 5, 6 and 8 hours after the administration, and anti-factor Xa activities in plasma were measured as the index of the absorbability of parnaparin sodium.

The results are shown in Table 10. As apparently shown in Table 10, Dog ID: S, G and Y respectively showed more than 0.6 U/mL, 0.3 U/mL and 1.0 U/mL of anti-factor Xa activity.

Table 10: Plasma anti-factor Xa activities after oral administration to dog

Dog ID	Dose of Parnaparin	Time (hr) after administration							
		0	1	2	3	4	5	6	8
S	5 mg/kg	-	-	-	+	++	+++	++	+
G		-	-	-	++	++	+	+	-
Y		-	-	++	+++	+++	+++	++	++

0.5U/mL ≦ Anti-factor Xa activity : +++
0.2U/mL ≦ Anti-factor Xa activity < 0.5U/mL : ++
0.1U/mL ≦ Anti-factor Xa activity < 0.2U/mL : +
0.1U/mL > Anti-factor Xa activity : -

Experimental Example E

75 mg of parnaparin sodium and 2.5 mg of citric acid were dissolved in 0.33 mL of purified water and to this solution was added 0.67 mL of caprylocaproyl macrogol glycerides, and the resulting solution was well stirred. Then, 100 mg of lauroyl macrogol-32 glycerides (EP) was added and the mixture was well stirred under 45°C to obtain the clear solution. The resulting solution was filled into the film obtained by Example 3 and sealed with commercially available adhesive agent to obtain the patch formulations (having 7.0 x 1.2 cm or 7.5 x 1.2 cm in size).

Beagle dogs weighing from 8.5 to 14.0 kg were treated with abdominal operation and the patches obtained above were applied to the mucosal surface of the ileum at the dose of 2 mg/kg or 5 mg/kg per patches. The blood samples were collected from the jugular vein before the administration, and at the predetermined time after the administration, and anti-factor Xa activities in plasma were measured as the index of the absorbability of parnaparin sodium.

As the results, all tested dogs showed more than 1.0 U/mL of anti-factor Xa activity.

Experimental Example F

450 mg of parnaparin sodium and 15 mg of citric acid were dissolved in 2 mL of purified water and to this solution was added 4 mL of caprylocaproyl macrogolglycerides, and the resulting solution was well stirred. Then, 600 mg of lauroyl macrogol-32 glycerides (EP) was added and the mixture was well stirred under 45°C to obtain the clear solution.

Then, 2 mL of above obtained clear solution was adsorbed to 1 g of magnesium aluminate metasilicate (Neusilin® US_2 or UFL_2; Fuji Chemical Industry, Co., Ltd.) in a mortar and the mixture was well mixed with a pestle to obtain powder. To this powder were added 150 mg of micro-crystalline cellulose and 300 mg of sodium starch glycollate, and the resulting mixture was well mixed and screened with sieve #60 to obtain the powder.

The powder obtained above was administered into the duodenum of Wistar rats weighing from 370 to 510 g so as to administer 2 mg/kg of parnaparin sodium, and anti-factor Xa activities in plasma were measured as the index of the absorbability of parnaparin sodium.

As the results, all tested rats showed increases in plasma anti-factor Xa activities.

Further, the obtained powder was pressed by mean of the tableting machine under 100 kg to prepare the tablet formulations. The obtained tablet was administered at the ileum of Wistar rats weighing from 310 to 390 g so as to administer 2 mg/kg of parnaparin sodium, and anti-factor Xa activities in plasma were measured as the index of the absorbability of parnaparin sodium.

As the results, all tested rats showed increasing of plasma anti-factor Xa activities.

Example 1

10 g of stearyl penta(tetra)glycerides [Trade name: PS-310; Sakamoto Yakuhin Kogyo Co., Ltd.] was heated and fused at 85°C. To

this fused solution were added 10 g of parnaparin sodium, 10 g of caprylic acid and 2 g of acrylic acid polymer [Trade name: Carbopol® 934P; The BF Goodrich, Ltd.], and the mixture was stirred for dispersing at 80°C for 15 minutes. Then, the resulting mixture was dropped at 10 g/minute on the aluminum disc with diameter of 15 cm rotating with 1,500 rpm to obtain spherical fine granules. The obtained fine granules were treated with enteric coating and filled in capsule.

Example 2

50 mg of parnaparin sodium and 2.5 mg of citric acid were dissolved in 0.56 mL of distilled water, and to this solution was added 1.1 mL of Labrasol® to obtain a clear solution.

On the contrary, an inside wall of a commercially available gelatin capsule was coated with a mixture solution of Eudragit® S100 in methylene chloride-methanol (4:1) to have a thickness of about 40 μm to obtain an enteric capsule. Then, the clear solution preparing above was separately injected into three enteric capsules thus obtained, and each hole was sealed with condensed enteric polymer to obtain the enteric capsule formulations.

Example 3

550 mg of ethylcellulose and 50 μL of triethylamino citrate were dissolved in 5 mL of a mixture solution of methylene chloride and methanol (4:1). The resulting solution was poured onto a Tefron® plate having 10 x 10 cm in size, and the solvent was evaporated to form an ethylcellulose-film (EC film). The EC film was put on a metal mold having projections of 200 μm maximum diameter and 80 μm height, and pressed at 75°C for 10 minutes. The film was then cooled to room temperature to form an EC film having the micro-container form, and about 0.5 μL of parnaparin sodium-Labrasol® solution obtained in the Example 2 was filled into the

micro-container.

In order to prepare a surface layer film, 300 mg of enteric HP-55 polymer and 50 μg of citric acid were dissolved in 10 μg of a mixture solution of methylene chloride and methanol (4:1). The resulting solution was poured onto a Tefron® plate having 15 x 15 cm in size, and the solvent was evaporated to form HP-55 film.

As glue for adhesion, 0.8 g of Hiviswako® 103 (Wako Pure Chemical Industries, Ltd.), 2 mL of distilled water and 250 μL of polyethylene glycol 400 were stirred in a mortar with a pestle. The glue for the adhesion was spread on the HP-55 film and stuck to the EC film having micro-container filled with the drugs, i.e., parnaparin sodium and Labrasol®.

Under the microscope, the resulting layered film was cut around the micro-containers into squares about 500 x 500 μm or circles about 500 μm in diameter to give un-uniformed microcapsule formulations having three-layers structure.

Example 4

Cellulose membrane having 3 mm diameter was attached onto the ethylcellolose film having a thickness of about 35 μm under heating and pressing. Then, 2 μL of the mixture solution of parnaparin sodium and Labrasol® prepared in the Example 2 was adsorbed the cellulose membrane.

In order to prepare an enteric protecting layer film, glue made of Hiviswako® 103 (Wako Pure Chemical Industries, Ltd.) was spread onto the surface of an enteric film having a thickness of about 30 μm made of HP-55, Eudragit® S100 or L100. Then, the treated film was further coated with the same enteric film to obtain the enteric protecting layer film having three-layers structure.

Then, the cellulose membrane was press-coated with the above enteric protecting layer film and ethylcellulose film so as to put the cellulose membrane between the enteric protecting layer film

and ethylcellulose film. The resulting layered film was cut into small pieces having ring pattern of diameter about 3.5 mm in size. The pieces were filled into an enteric polymer capsules.

Example 5

The baggy type container, 3 mm diameter and 0.5 mm depth, made of cellulose acetate film coated with gelatin layer was prepared from the heat-laminated film of the cellulose acetate film and gelatin film. Then, 2 μL of the mixture solution of parnaparin sodium and Labrasol® prepared in the Example 2 was filled in the container and was covered with an enteric film coated with gelatin layer. An open area of the container was sealed by means of heat pressing, and further cut into the film form having a diameter of 3.6 mm. The resulting films were filled into enteric capsules to obtain the capsule formulations by the same manner as described in the Example 3.

Example 6

75 mg of parnaparin sodium and 2.5 mg of citric acid were dissolved in 0.33 mL of purified water and to this solution was added 0.67 mL of caprylocaproyl macrogolglycerides, and the resulting solution was well stirred. Then, 100 mg of lauroyl macrogol-32 glycerides (EP) was added and the mixture was well stirred under 45°C to obtain the clear solution.

Then lactose and microcrystalline cellulose (1:3) were well mixed in a mortar with a pestle. To 450 mg of this mixture was added 0.55 mL of above obtained clear solution, and the resulting mixture was well mixed in a mortar with a pestle to obtain a wet mass. The mass was passed through a sieve to prepare granules, and the obtained granules were filled into an enteric capsule basically consisting of Eudragit® S100, Eudragit® L100 or HP-55, and the capsule was sealed, or filled into gelatin capsule and this gelatin capsule

was enteric-coated with Eudragit® S100, Eudragit® L100 or HP-55 after sealing to obtain the enteric capsule formulations.

Example 7

Sucrose and citric acid (1:1) were well mixed in a mortar with a pestle. To 500 mg of this mixture was added 0.55 mL of the clear solution obtained in the Example 6, and the resulting mixture was well mixed in a mortar with a pestle to obtain a wet mass. The mass was passed through a sieve to prepare granules, and the obtained granules were filled into an enteric capsule basically consisting of Eudragit® S100, Eudragit® L100 or HP-55, and the capsule was sealed, or filled into gelatin capsule and this gelatin capsule was enteric-coated with Eudragit® S100, Eudragit® L100 or HP-55 after sealing to obtain the enteric capsule formulations.

Example 8

450 mg of parnaparin sodium and 15 mg of citric acid were dissolved in 2 mL of purified water and to this solution was added 4 mL of caprylocaproyl macrogolglycerides, and the resulting solution was well stirred. Then, 600 mg of lauroyl macrogol-32 glycerides (EP) was added and the mixture was well stirred under 45°C to obtain the clear solution.

Then, 2 mL of above obtained clear solution was adsorbed to 1 g of magnesium aluminate metasilicate (Neusilin® US_2 or UFL_2; Fuji Chemical Industry, Co., Ltd.) in a mortar and the mixture was well mixed with a pestle to obtain powder. To this powder were added 150 mg of micro-crystalline cellulose and 300 mg of sodium starch glycollate, and the resulting mixture was well mixed and screened with sieve #60 to obtain the powder.

The powder obtained above was pressed by mean of the tableting machine under 100 kg to prepare the tablet formulations.

INDUSTRIAL APPLICABILITY

According to the present invention, mucopolysaccharide, which has not been administered orally, can be administered orally by the enteric formulation containing said mucopolysaccharide and absorption enhancer thereof.

CLAIMS

1. A formulation containing mucopolysaccharide and absorption enhancer thereof, wherein said formulation is characterized by its enteric formulation to release the mucopolysaccharide and absorption enhancer thereof from the formulation when said formulation reaches at the middle to lower part of small intestine.

2. The formulation according to claim 1, wherein the formulation contains mucosa adhesive substance which adheres to mucosal membrane of the middle to lower part of small intestine and retains at the middle to lower part of small intestine for a long period of time.

3. The formulation according to any one of claims 1 and 2, in which said mucosa adhesive substance is acidic polymer having carboxylic group or sulfonic group or salt thereof.

4. The formulation according to any one of claims 1 to 3 which comprises a basic layer having a drug container, a surface layer sealing said drug container and a drug-carrying layer for containing mucopolysaccharide and absorption enhancer thereof in which the basic layer consists of water-insoluble polymer, the surface layer consists of enteric substances, and the drug container consists of said basic layer and said surface layer.

5. The formulation according to any one of claims 1 to 4, in which said mucopolysaccharide is having the average molecular weight from 3,000 to 20,000.

6. The formulation according to any one of claims 1 to 5, in which said mucopolysaccharide is having the average molecular weight from 5,000 to 9,000.

7. The formulation according to any one of claims 1 to 6, in which said mucopolysaccharide is selected from the group consisting of heparin, low molecular weight heparin, hyaluronic acid, chondroitin sulfate, dermatan sulfate, heparan sulfate, ketaran sulfate, and glucurono-2-amino-2-deoxy-glycoglycan sulfate.

8. The formulation according to any one of claims 1 to 6, in which said mucopolysaccharide is heparin or low molecular weight heparin.

9. The formulation according to any one of claims 1 to 8, in which said formulation contains mucopolysaccharide in the form of aqueous solution.

10. The formulation according to any one of claims 1 to 9, in which said formulation contains mucopolysaccharide in the form of lyophilized powder thereof.

11. The formulation according to any one of claims 1 to 8, in which said absorption enhancer for mucopolysaccharide is selected from the group consisting of fatty acid having 6 to 14 carbon atoms, or salt, ester and amide derivative thereof; steroid carboxylic acid, or salt, ester and amide derivative thereof; and aliphatic poly-basic acid, or salt, ester and amide derivative thereof; or mixture thereof.

12. The formulation according to any one of claims 1 to 8, in which said absorption enhancer for mucopolysaccharide is selected from the group consisting of deoxycholic acid, capric acid, edetic acid, caprylocaproyl macrogolglycerides and lauroyl macrogol-32 glycerides.

13. The formulation according to any one of claims 1 to 8, in which said absorption enhancer for mucopolysaccharide is a mixture of caprylocaproyl macrogolglycerides and lauroyl macrogol-32 glycerides.

14. The formulation according to any one of claims 1 to 8, in which said absorption enhancer for mucopolysaccharide is a mixture of caprylocaproyl macrogolglycerides and citric acid.

15. The formulation according to any one of claims 1 to 8, in which said absorption enhancer for mucopolysaccharide is caprylocaproyl macrogolglycerides.

16. The formulation according to any one of claims 2 to 12, in which said mucosa adhesive substance is acrylic acid polymer or salt thereof.

17. The formulation according to any one of claims 1 to 16, wherein said formulation is characterized by its enteric formulation containing powdery mixture of mucopolysaccharide and absorption enhancer thereof.

18. The formulation according to any one of claims 1 to 16, wherein said formulation is tablet obtained by tableting powdery mixture of mucopolysaccharide and absorption enhancer thereof, or by tableting the granules prepared from the powdery mixture of

mucopolysaccharide and absorption enhancer thereof, and then enteric-coated.

19. The formulation according to any one of claims 1 to 16, wherein said formulation is capsule formulation containing the granules prepared from powdery mixture of mucopolysaccharide and absorption enhancer thereof and the resulting capsule is enteric-coated.

20. The formulation according to any one of claims 1 to 16, wherein said formulation is enteric capsule formulation containing the granules prepared from the powdery mixture of mucopolysaccharide and absorption enhancer thereof.